

REVIEW ARTICLE

Respiratory syncytial virus–Host interaction in the pathogenesis of bronchiolitis and its impact on respiratory morbidity in later life

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Abstract

Respiratory syncytial virus (RSV) is the most common agent of severe airway disease in infants and young children. Large epidemiologic studies have demonstrated a clear relationship between RSV infection and subsequent recurrent wheezing and asthma into childhood, thought to be predominantly related to long-term changes in neuroimmune control of airway tone rather than to allergic sensitization. These changes appear to be governed by the severity of the first RSV infection in infancy which in turn depends on viral characteristics and load, but perhaps as importantly, on the genetic susceptibility and on the constitutional characteristic of the host. A variety of viral and host factors and their interplay modify the efficiency of the response to infection, including viral replication and the magnitude of structural and functional damage to the respiratory structures, and ultimately the extent, severity, and duration of subsequent wheezing.

Respiratory syncytial virus (RSV) is the single most important cause of acute lower respiratory tract infection (LTRI) in infancy, with nearly all children being seropositive by the age of 2 years (1). While the majority of affected children is asymptomatic or has only mild illness, significant morbidity is exhibited in 2–3% of affected infants resulting in hospitalization almost universally due to severe respiratory symptoms (2, 3). Although a number of constitutional and environmental risk factors have been identified (4, 5), epidemiologic data show that the vast majority of infants hospitalized for this condition does not constitute an “at-risk” group (4–7), suggesting that viral or host factors, not included in the classical risk factors, may be accountable for disease severity and play a putative role

in the magnitude of the subsequent respiratory morbidity in later life (7).

RSV bronchiolitis and recurrent wheezing

Cohort and long-term prospective case-control studies have shown that RSV bronchiolitis is associated with an increased risk of developing recurrent wheeze and asthma in later life and that this link might be related to the severity of the first RSV infection (8). Subjects who experience an increased risk of wheezing and asthma, reduced lung function, and irreversible airway obstruction up to age 18–31 years are predominantly those who received hospital-based care for RSV infection

Abbreviations

AP-1, activator protein-1; BAL, bronchoalveolar lavage; BMSCL, bone marrow stromal cell line; FLT-3, fms-like tyrosine kinase; IFN, interferon; IP-10, interferon gamma-induced protein 10; JAK, Janus kinase; JNK, c-Jun N-terminal protein kinase; MCP-1, monocyte chemoattractant protein-1; MDA5, melanoma differentiation-associated gene 5; MHC, major histocompatibility complex; MIP, macrophage inflammatory protein; NANC, non-adrenergic, non-cholinergic; NF- κ B, nuclear factor kappa-light-chain-enhancer of activated B cells; NGR, nerve growth factor; NK cells, natural killer cells; NK₁, neurokinin 1 receptor; p38 MAPK, p38 mitogen-activated protein kinase (I); p75^{NTR}, p75 neurotrophin receptor; PAMP, pathogen-associated molecular pattern; PI 3-Kinase, phosphoinositide 3-kinase; PMN, polymorphonuclear leukocyte; PRRs, pattern recognition receptors; RANTES, regulated on activation, normal T cell expressed and secreted; RIG-I, intracellular retinoic acid-inducible gene I; STAT, signal transducer and activator of transcription; SubP, substance P; TGF- β , transforming growth factor- β .

within the first months of life (9–13). The hypothesis of the key pathogenetic role of RSV in childhood wheeze is supported by the demonstration that prevention or delay of RSV infection reduces its prevalence. A placebo-controlled trial demonstrated that palivizumab prophylaxis in late-preterm infants not only lowers the incidence of RSV-related infection and hospitalization but significantly reduced the number of subsequent wheezing days during the first year of life, including outside RSV season (14). Similarly, in preterm infants, a prospective multicenter study showed that palivizumab prophylaxis significantly decreased physician-diagnosed recurrent wheezing through age 2–5 years (15). This study also showed that palivizumab prophylaxis was effective mainly in subjects without a family history of atopy or food allergies, suggesting that RSV infection may represent a risk factor for future respiratory morbidity, independent from a genetic predisposition to atopy (15). A close evaluation of the complex interactions between RSV and the host is a key step in uncovering factors that could explain the pathogenicity of RSV infections also in “not-at-risk” populations (16–20).

RSV structure and function

RSV is a single-stranded RNA virus of the Paramyxoviridae family whose genome includes 10 genes encoding 11 proteins: three transmembrane surface glycoproteins (the attachment G protein, the fusion F protein, and the small hydrophobic SH protein), three proteins associate with the genomic RNA to form the nucleocapsid (the large L polymerase subunit, the N protein, and the phospho P protein), two non-structural proteins (the NS1 and NS2 proteins), two transcription and replication factors (M2-1 and M2-2), and one unglycosylated matrix M protein (Fig. 1) (16, 19). Proteins F and G are key factors for virus attachment, fusion, and RSV nucleocapsid release into the cytoplasm, whereas viral transcription and subsequent replication require the L, N, P, M-1, and M-2 proteins that, together with the genomic RNA, form the ribonucleoprotein (RNP) complex. The synthesis of positive and negative-sense genomic RNA (“replicase” activity), as well

as subgenomic mRNAs (“transcriptase” activity), is controlled by the RNA polymerase, attached to the RNP complex. The NS1 and NS2 proteins, not essential for viral replication, and the sG protein (the soluble form released during viral replication) interfere with cell defense mechanisms by downregulating antiviral response. The SH protein has the dual function of permeabilizing cell membrane and preventing apoptosis of the infected cells (20).

The innate immune response to RSV infection

Airway epithelial cells (AECs) at the bronchial, bronchiolar, and alveolar levels are the main targets for RSV infection (21). The innate immune response is activated via recognition by pattern recognition receptors (PRRs) of the surface proteins, RSV RNA, and proteins produced during the viral lifecycle. The TLR4/CD14 complex is the main extracellular receptor recognizing RSV through binding with the F protein, while intracytoplasmic RIG-I, MDA5, and TLR3 PPRs recognize RSV transcripts and viral replication intermediates (16–19). Activation of a variety of transcription factors occurs, including NF- κ B, AP-1, JNK, JAK, p38 MAPK, and STAT signaling pathways, followed by the production of cytokines, chemokines, and type I and type III IFNs, all geared to mount an effective antiviral reaction (16–19, 22, 23). These various molecules are grouped by their relative roles in the virus-host interplay in Fig. 2, as further outlined in the following paragraphs. TNF- α , CXCL8/IL-8, and ITAC/CXCL11 recruit and activate PMNs and NK cells that destroy the infected AECs in the first days of infection, while type I IFNs display potent antiviral activity, reducing protein synthesis and upregulating pro-apoptotic factors. Apoptosis of the infected cell is a key protective mechanism in that it aborts viral replication, prevents both spread of infection with the release of a large numbers of progeny viruses, and further production of proinflammatory mediators (16–19, 22, 23). RSV infection of AECs promotes also the secretion of other chemokines, such as MIP-1 α /CCL3, MIP-1 β /CCL4, MIP-2, IP-10/CXCL10, and MCP-1/CCL2, all of which induce recruitment and activation

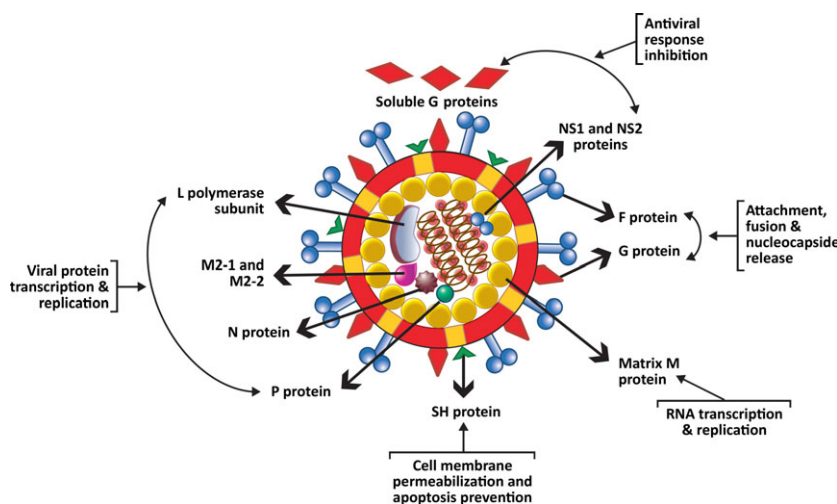


Figure 1 RSV proteins by functions.

The three transmembrane surface glycoproteins (the G, F, and SH), the three proteins associate with the genomic RNA (the L, N, and P), the two non-structural proteins (the NS1 and NS2), the two transcription and RNA replication factors (M2-1 and M2-2), and the unglycosylated matrix M protein are all involved in viral RNA and protein transcription and replication and in the inhibition of the antiviral responses.

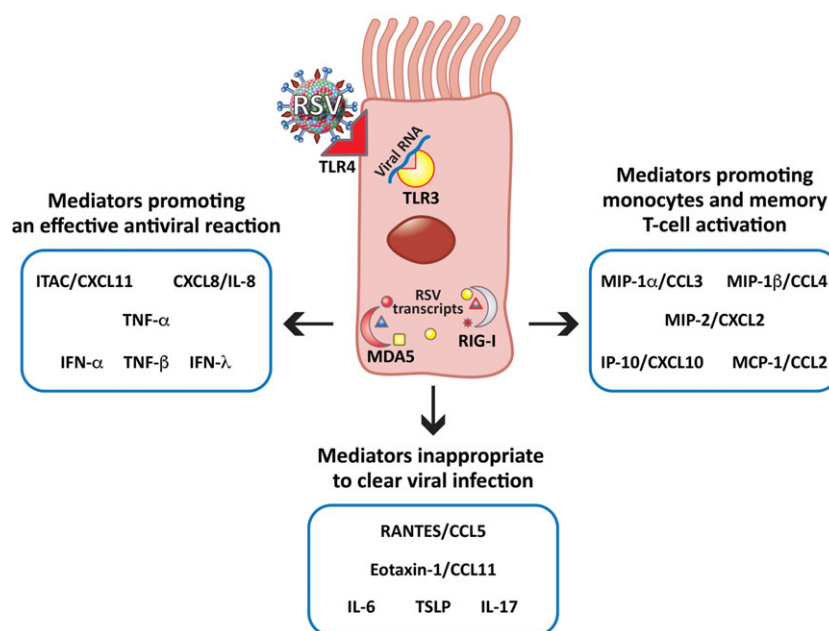


Figure 2 The immune response of airway epithelial cells (AECs) to RSV infection. Depending on the modulation of the response to RSV infection, AECs release mediators that can promote an effective antiviral reaction or favor an inappropriate clearing of the viral infection.

of monocytes and memory T cells (24). Along these protective processes, however, recognition of RSV transcripts and viral replication intermediates by PRRs may also induce the production of cytokines and chemokines involved in the recruitment/activation of eosinophils, basophils, and mast cells, exemplified by RANTES/CCL5, eotaxin-1/CCL11, IL-6, TSLP, and IL-17C. These products have no role in clearing viral infection, yet, by promoting Th0 cell polarization toward a robust Th2 phenotype, aggravate the pernicious viral infection by contributing to airway damage (22–26).

Viral and host factors that may downregulate the innate anti-RSV immune response

RSV utilizes the G, NS1, and NS2 proteins to interfere with host cell defense mechanisms and inhibit antiviral responses (16). The G protein has been considered the most “antigenic” RSV pathogen-associated molecular pattern (PAMP) and therefore most deeply involved in mediating host reaction (27). G protein genes can be classified into A and B subgroups; it was initially suggested that the GA3 genotype was a key factor for more severe infection-related morbidity; however, recent reports indicate that viral load, rather than variations in circulating strains, is the pathogenetic determinant (27, 28). Being highly glycosylated and characterized by broad variability, the G protein impedes immune recognition and allows easy escape from neutralizing antibodies (18) (Table 1). The G protein can also counteract the antiviral activities of the host cells by manipulating the synthesis of microRNA (miRNA), small single-stranded RNA molecules regulating gene expression that are emerging as important factors in determining host response in RSV infection (29, 30). In a study in AEC line, through the induction of let-7f miRNA transcriptional activity, RSV G protein significantly downregulated three “cell

proliferation and survival” genes (CCND1, DYRK2, and ELF4) and one “chemokine” gene (CCL7/MCP3), with the effect of delaying viral clearance (30). Imbalance in miRNA production and modulation of their transcription can be associated with RSV infection severity, decreasing apoptotic signals and inhibiting TLR4 synthesis and function (31–33). In addition to the effects on miRNA, the G protein in its soluble form inhibits antiviral responses by binding RSV-specific antibodies (thus reducing their available concentrations for RSV neutralization) and acting as a TLR antagonist (34) (Table 1). Finally, the NS1 and NS2 proteins, interacting with the intracytoplasmic PRRs RIG-I and MDA5, downregulate type I IFN production by modulating three key signaling molecules: (i) the STAT2 transcription factor, essential for IFN-inducible antiviral genes, (ii) the TNF receptor-associated factor-3, an integrator of multiple IFN-inducing signal molecules, (iii) the inhibitor- κ B kinase ϵ , that activates IFN regulatory factor-3 (35, 36). Severe RSV infection has been related to single nucleotide polymorphisms (SNPs) in genes that influence the innate host defenses (Table 1) (37). Impaired responses to RSV bronchiolitis severity and increased risk for hospitalization have been associated with TLR4 SNPs (38) and human bronchial epithelial cells (BECs) expressing the TLR4 gene ²⁹⁹Gly and the ³⁹⁹Ile alleles failed to efficiently activate NF- κ B signaling, with decreased production of IFNs, IL-8, IL-12p35, IL-18, CCL8, and TNF- α , in response to RSV infection (39). Genetic factors may also explain the conflicting results on the efficacy of vitamin D (VD), the fat-soluble secosteroid able to potentiate the secretion of type 1 interferon, of chemokines (CXCL8 and CXCL10) and cytokines (TNF- α and IL-6) from human BECs, in response to RSV infection (40). Indeed, in children from South Africa, the Thr1Meth, but not the JUN (c.750G/A) SNP allele of the VD receptor, was identified as a risk factor for severe RSV disease (41) and genotype analysis

performed in different populations showed positive relationships between VD receptor FokI (rs2228570) SNP and RSV disease severity (42). Surfactant protein (SP)-A and Surfactant protein-D gene polymorphisms have also been proposed to explain host susceptibility to respiratory disease (43). In a Chilean study, SP-D Thr(11) and Thr(160) alleles were more commonly detected in RSV infants, as compared to normal subjects, and their frequency was even higher in infants with severe disease. In that study, an increased host susceptibility to severe disease was shown for the associations of Thr160 allele with the 1A0 variant of SP-A2 and of Thr11-Thr160 with the 1A0 variant, while the association of the Ala160 allele with the 6A2 variant of SP-A1 appeared to be protective (43). These findings were only partially confirmed by other authors who studied different populations (44, 45), indicating the need for local studies to identify genetic biomarkers of severity in different countries and ethnic groups.

The adaptive immune response to RSV infection

In addition to bronchial, bronchiolar, and alveolar epithelium, RSV is also infecting airway dendritic cells (DCs) (17, 19, 25). Among the heterogeneous DC lineages, plasmacytoid DCs (pDCs) and myeloid DCs (mDCs) predominate at the airway levels and synergistically induce efficient antiviral immune responses (46, 47). By secreting type I IFNs, pDCs promote antiviral effects by acting over a wider range of cell types, while mDCs stimulate CD4⁺ and CD8⁺ T cells to induce efficient adaptive immune responses against viruses (47). Upon recognition of PAMPs, DCs migrate to lymphoid tissues. RSV-infected mDCs present viral antigens to specific helper CD4⁺ T cells and depending on the presenting stimulus and the immunological environment, naive T cells differentiate into specific cell subsets (Fig. 3) (16–20, 25). *Th1 polarization*, driven by IL-12 secreting mDCs, promotes activation of CD4⁺ T cells. IFN- γ production by CD4⁺ T cells activates CD8⁺ cytotoxic T cells and natural killer (NK) cells that clear virus-infected cells, and stimulate macrophage phagocytic activity to endorse dead cell clearance (16–20, 25). In contrast, the induction of a prevalent *Th2-type response*, with the production of IL-4, IL-5, and IL-13, results in a less robust and less efficient reaction to the infection, clinically presenting as increased disease severity and risk for future recurrent wheezing (16–20, 25). While NK cell activity contributes to a rapid termination of virus infection during the early stage of infection, its eradication from the lung is mediated by the virus-specific CD8⁺ T cells that kill virally infected cells by direct cytolysis through perforin/granzyme-mediated lysis (16–20, 25, 48). CD8⁺ cells may also suppress the development of virus-specific Th2-dominated immune responses and eosinophilia through mechanisms that include IFN- γ release (22, 49). Two other T-cell subsets are involved in the response to RSV infection: regulatory T cells (Treg) and the interleukin IL-17-producing T helper cells (Th17) (50). In addition to regulating autoimmunity and antitumor immunity, Treg cells are global regulators of adaptive immune responses. In murine models of RSV infection, Treg cells have proven to promote

Table 1 Viral and cellular components affecting the innate anti-RSV immune response

	Functions
Viral components	
G-protein	Impedes immune recognition by neutralizing antibodies because of structural variability and high glycosylation Counteracts antiviral activities by manipulating miRNA synthesis by AECs
sG-protein	Binding RSV-specific antibodies, reduce their concentrations available for RSV neutralization Acting as a TLR antagonist downregulates inflammatory responses
NS1 and NS2 proteins	Suppress type I IFN production modulating signaling molecules
Host cell components	
TLR4 SNPs	Increase risk of severe bronchiolitis by impairing immune responses to RSV
Vitamin D receptor SNPs	Identified as risk factors for RSV severe disease Associated with increased host susceptibility to respiratory disorders
Surfactant protein (SP)-A and -D SNPs	Different effects on RSV infection susceptibility and/or severity in different countries and ethnic groups

SNPs, single nucleotide polymorphisms.

efficient viral clearance and modulate the innate and adaptive immune responses by: (i) activation of NK cells and neutrophils, (ii) early recruitment of CD8⁺ cytotoxic T cells from mediastinal lymph nodes to the lungs, (iii) prevention of an excessive RSV-specific CD4⁺ and CD8⁺ T-cell response, (iv) limitation of an inefficient Th2-type immune responses through the production of inhibitory cytokines on Th2 response, such as IL-10 and TGF- β (50–52). These positive infection controlling effects of Treg cells can, however, be counterbalanced by Th17 cells (51). Due to the ubiquity of its receptor on epithelial and endothelial cells, on monocytes and macrophages, IL-17 is capable of inducing a powerful proinflammatory response that includes downregulation of the CD8⁺ cytotoxic T-cell functions (50, 53). IL-17 was found in elevated concentrations in the lower airways of infants with severe RSV bronchiolitis, where it can cause diminished viral clearance, and exaggerated mucus production and neutrophilic infiltration (50, 53). As Treg differentiation may be inhibited by Th17 cells and Treg cells can differentiate into Th17 cells in response to cytokines, it has been hypothesized that an imbalance between these two T-cell subsets, presumably with dominance of Th17, could impact on RSV pathogenesis (50). In lymphoid tissues, antigen presentation by RSV-infected mDCs can also lead to B-cell proliferation, differentiation, and immunoglobulin production (54). B cells and IgM-, IgG-, and IgA-positive plasma cells are then recruited to the airways at the peak of RSV illness and antibody production occurs under the influence B-cell trophic and stimulating factors (54). In mice, RSV-specific antibody

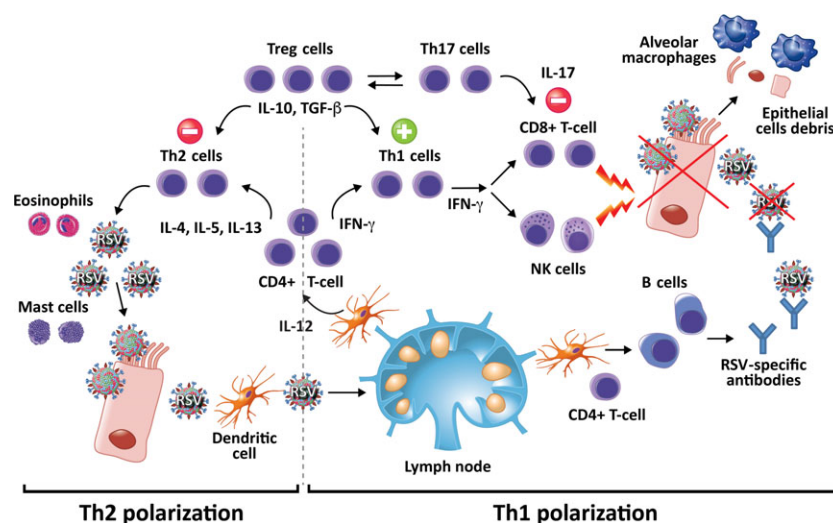


Figure 3 The adaptive immune response to RSV infection. Upon recognition of RSV antigens, DCs migrate to lymphoid tissues where they present viral antigens to specific helper CD4+ T cells. Depending on the immunological environment, naive T cells differentiate into Th1 or Th2 cells. Th1 polarization promotes IFN- γ production with activation of CD8+ cytotoxic T cells and NK cells to clear virus-infected cells. In contrast, Th2 polarization results in an inefficient immune response, increased disease severity, and risk for future recurrent wheezing. Treg cells, whose activity is counterbalanced by Th17 cells, prevent disproportionate or ineffective responses to infection. In lymphoid tissues, following antigen presentation by RSV-infected mDCs, B-cell proliferation and differentiation arise, followed by RSV-specific IgM, IgG, and IgA production.

production after nasal infection is initially restricted to the local regional lymphoid tissues and, only some 14 days later, becomes detectable in the respiratory-associated lymphoid tissues, in the tracheal lamina propria and in the lung (55). B cells generated in the airways, however, are short-lived and do not undergo maturation affinity (54).

Viral and host factors that may negatively affect the adaptive anti-RSV immune response

Inefficiency of antiviral responses may be explained by interference of RSV or host factors with some of the DC functions outlined above and summarized in Table 2. A lower efficiency of pDCs in early life may contribute to increased susceptibility to severe RSV disease in infants. When infected with RSV, immature, cord blood stem cell-derived DCs showed decreased capacity to induce IFN- γ generation by naïve T cells, which corrected as DC matured (56). As compared to adults, a greatly attenuated IFN-I production at birth and in childhood was shown following RSV infection of peripheral blood-derived DCs (57). In newborn, but not in adult mice, the response to intranasal RSV infection was dominated by the CD103+ DC subpopulation (58, 59). These cells were less effective in antigen uptake and processing, generated attenuated CD8+ T-cell responses and showed an age-dependent downregulation of the expression of the co-stimulatory molecule CD86 (59). In preterm intubated and ventilated infants with severe RSV bronchiolitis, impaired pDCs migration from the bone marrow to the lower airways was detected by BAL, despite normal or elevated pDC percentages in blood, suggesting a selective dysfunction of cell migration (60). Indeed, in neonatal mice with severe RSV

Table 2 Host components affecting the adaptive anti-RSV immune response

Components	Functions
Dendritic cells	Reduced mobilization of precursors from the bone marrow Dysfunction of cell migration from blood to the airways in infants Weak engagement of the IFN I pathway and impaired capacity to stimulate CD4+ T-cell proliferation
T-cells	Reduced number of circulating memory helper-inducer T-cells with higher percentage of naïve suppressor-inducer subpopulations in infants Tendency to react to infection with a Th2 response, with inadequate activation of cytotoxic CD8+ cells
B-cells	Deficiency in neutralizing antigen-specific antibody production following RSV infection possibly due to the presence of maternally derived antibody
Cytokines	Promoter polymorphisms in the RANTES, the 5q31 Th2 cytokine cluster genes, the IL-1RL1, the IL-9 and the IL-10 family members genes, all associated with the severity of RSV disease in infancy and/or the occurrence of recurrent wheeze in later life

infection, poor mobilization of DC precursors from bone marrow was described, associated with weak activation of the IFN-I pathway, dysfunctions reversed by the administration of FLT3 ligand, a growth factor that stimulates hematopoietic cell proliferation (61). Twelve and 21 days following RSV

infection, the percentage of mDCs precursors was deeply depleted not only in the young but also in lungs of adult mice and these cells did not expand and mature into mDCs *ex vivo* in response to growth factors (62). This low number of local DC precursors that follows RSV infection may explain the increased susceptibility to subsequent respiratory infections as a sequel of RSV bronchiolitis. Recent contributions demonstrated the important role of autophagy a cellular activity that facilitates self-digestion of misfolded or unused protein and cellular debris that impacts in a variety of cellular processes. Autophagy may be particularly important in RSV infection, not only because it promotes infected cell apoptosis but also because it facilitates DC maturation and presentation of viral antigens by DC to T cells (63). Figure 4 summarizes the sequence of events RSV cytoplasmic entry induces autophagosome formation. Following fusion with lysosome, RSV transcripts and viral replication intermediates are degraded and released by the phagolysosome to be recognized by the intracytoplasmic RIG-I, MDA5, and TLR3 PPRs (63). Mice deficient in the autophagy-associated proteins Beclin-1, SIRT1, and Map1-LC3b showed impaired immune response to RSV. Upon RSV infection, Beclin-1(±) mice DCs showed decreased MHC-class-II molecule expression and IFN- γ production, in comparison with wild-type controls (64). Beclin-1(±) mice lung histology following RSV infection showed increased Th2 cytokine generation, mucus secretion, and lung infiltration by eosinophils (64). In other autophagy-impaired mice, deficient in the autophagy-associated protein Map1-LC3b, RSV infection was associated with inhibition of Treg activation and excessive production of IL-17a, and hence increased lung pathology (65). Finally, elevated viral load and enhanced Th2 cytokine production with resulting more severe lung pathology and increased mucus production were observed in RSV-infected mice treated with a selective SIRT1 inhibitor (66). A defective helper T-cell response with a Th1 over a Th2 polarization of the immune response is thought to play a determinant role in the control of RSV infection (Table 2). In infants with RSV bronchiolitis, high fraction of the naive suppressor-inducer subpopulation (CD4+/CD45RA+) and a low fraction of the reciprocal memory helper-inducer

subpopulation (CD4+/CD29+) were detected (Table 2) (67). Data from animal models suggest that the T-cell response may be biased toward a Th1-type response when primed by the RSV F protein, while the G protein favors a Th2 response (68–70). Five days after intranasal challenge, mice sensitized to G protein produced high levels of IL-4 and IL-5 and developed extensive pulmonary eosinophilia, while mice sensitized to F protein produced IL-2 and developed a mononuclear cell infiltration (71). The G protein contains a CX3C chemokine motif that can block IFN- γ secretion by RSV-specific T cells and reduce the frequency of lung CX3CR1+/CD4+ and CX3CR1+/CD8+ T cells, a major cytotoxic component in RSV infection (72). Immunization of Balb/c mice with RSV G protein was associated with failure to elicit any detectable RSV-specific CD8+ T-cell cytolytic response but with the oligoclonal expansion of the V β 14+, CD4+ T-cell population, that favors tissue injury (73). In the same strain of mice, challenge with the RSV G protein increased pulmonary levels of Substance P (SubP), a neuropeptide involved in the pathogenesis of neurogenic inflammation and bronchomotor tone regulation (74). The preferential production of Th1 over Th2 cytokines in some individuals may be related to a genetic predisposition (75). To date, a number of genetic variations of different adaptive immunity genes were shown that may be associated in different patient populations with RSV disease severity in infancy and/or with recurrent wheezing later in life. Among these, genetic association studies of RSV bronchiolitis have identified associations for promoter polymorphisms in the IL-1RL1 (76), IL-10 (77, 78), RANTES (79, 80), 5q31 Th2 cytokine cluster (81), and IL-9 (82) genes. Finally, young age seems to be negatively affecting also immunoglobulin production against both RSV G- and F proteins. In children studied in an outpatient setting with their first RSV infection, a significant proportion of the younger infants (<9 month) developed low levels of serum and nasal-wash neutralizing IgA and IgG antibodies against the F and G protein (83). In addition, most of these infants also failed to develop a rise in serum or nasal-wash neutralizing antibody thus, the resulting convalescent sera titers were 8- to 10-fold lower than in the older patients (83). The presence of maternally derived antibody in the younger

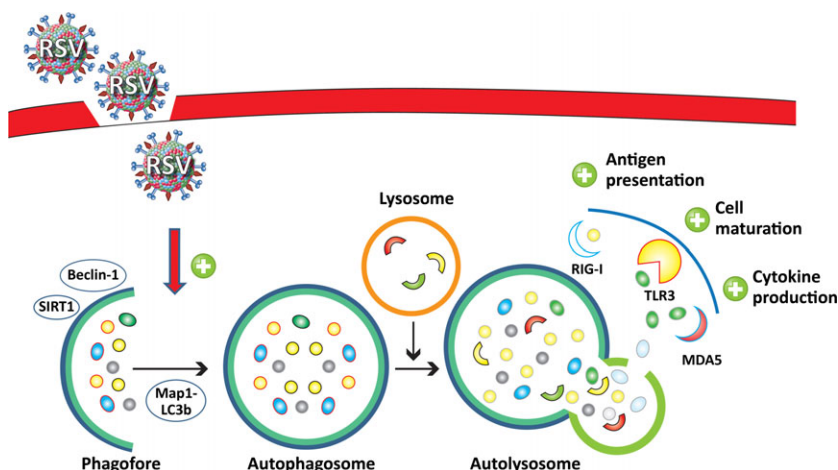


Figure 4 RSV cytoplasmic entry induces autophagosome formation. Following fusion with lysosome, RSV transcripts and viral replication intermediates are released by the phagolysosome to be recognized by the intracytoplasmic RIG-I, MDA5, and TLR3 PPRs.

infants may suppress the immune response to RSV infection and contribute to the severity of their initial infection and their failure to develop effective resistance to subsequent re-infection (54, 83).

RSV-induced neurogenic inflammation and extrapulmonary sites of infection

Airway hyperreactivity and inflammation observed during and after early-life RSV infection may be related, at least in part, to an abnormal neural control of airway smooth muscle tone induced by the virus and summarized in Fig. 5 (84). In a rat model of early-life RSV infection, upregulation of NGF and its TrkA and p75^{NTR} receptors and of the high-affinity neurokinin NK₁ receptor has been detected (85).

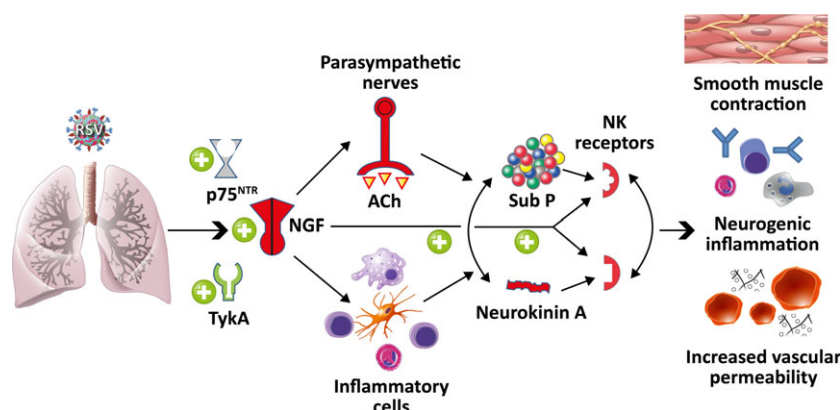
In addition to being a neurotrophic factor and inhibiting infected cell apoptosis, NGF acts as promoter of acetylcholine release and as a signaling molecule inducing neuropeptide production. These include neurokinin A and Substance P-mediated involved in the pathogenesis of neurogenic inflammation and in bronchomotor tone regulation (84). Elevation of NGF protein levels was also detected in BAL fluid of ventilated infants with acute RSV infection, and strong TrkA immunofluorescence was observed in BAL macrophages and AECs (85). The presence of a persistent, long-lasting activation of the neurogenic inflammation associated with airway pathology and bronchial hyperreactivity was demonstrated in mice up to 60 days after intranasal inoculation of RSV (86). In addition to increasing sensory fiber responsiveness, acetylcholine and proinflammatory peptides release during the acute phase of the infection, NGF may promote the long-term remodeling of NANC innervation in the respiratory tract by promoting overgrowth of neurites with higher SubP content (84–86). Moreover, RSV-induced NGF overexpression may worsen airway smooth muscle tone dysregulation through the reduction of catecholamine production, secondary to adrenal medulla cell differentiation into nerve cells (87). Indeed, the infection is not limited to the airways as RSV RNA has been detected in the blood (88), in myocardium (89), in cerebrospinal fluid (90), and liver (91). As shown for other pathogens (92), there is evidence that following exposure to RSV during viremic episodes, bone marrow may serve as a

sanctuary for latent infection, preventing a complete eradication of the virus. Indeed, in human BMSCs, sequences with virtually complete homology to the human RSV-A serotype genome were found in two-thirds of adult donors and in all pediatric donors (93). Furthermore, *in vitro* infection of BMSCs by RSV resulted in an overexpression of proinflammatory and mitogenic factors and decreased the ability of BMSCs to support B-cell maturation (93). Persistence of this latent infection could maintain a constant stimulation of the immune system and represent one mechanism underlying the respiratory sequelae of early-life RSV infection.

Possible impact of viral and host factors on subsequent respiratory morbidity

There is ample evidence for future respiratory consequences of RSV bronchiolitis in early life; the pathogenesis thereof, however, is still poorly understood. There is compelling evidence that infants in their early months of life, in whom the severity of RSV infections mandates hospitalization, have a subsequent increased risk of developing recurrent wheezing and asthma (9–13). This point is highlighted by a retrospective study in healthy term infants, demonstrating a dose-response relationship between the severity of bronchiolitis and the increasing odds for both early childhood asthma and asthma-specific morbidity (9). This relationship, that was not modified by a familial predisposition to asthma, highlights the pathogenetic role of the progressive severity of RSV-induced airway injury and future morbidity. This is possibly mediated by impairment in lung development and/or alteration in the maturation of the host immune system subsequent to the infection. The severity of the RSV disease in infancy may be related to viral load but also favored by SNPs in genes that affect the innate and the adaptive immune responses (37–45, 94). Age, both in terms of early gestational age and/or young chronological age, likely limits the efficiency of the host response to infection, also plays into the pathogenetic paradigm. (4–7). Indeed, a lower efficiency of immature DCs and of T and B lymphocytes, with a Th2 polarization, may adversely affect the adaptive immune response to the first RSV-induced bronchiolitis, but also lay the foundations for the increased susceptibility to subsequent respiratory infections (56–62). Elevated levels of Th2 cytokines

Figure 5 RSV-induced neurogenic inflammation. RSV infection upregulate the expression of NGF and its TrkA and p75^{NTR} receptors and of the high-affinity neurokinin NK receptors. NGF acts as promoter of acetylcholine release and as signaling molecule to induce the production of neurokinin A and SubP, involved in the pathogenesis of neurogenic inflammation and bronchomotor tone regulation.



and pro-inflammatory cytokines and chemokines were detected in nasopharyngeal aspirates (NPA) and BAL fluid from infants hospitalized with RSV bronchiolitis and a direct correlation found between IL-3 and IL-12p40 levels and the development of recurrent wheezing later in life (95). Moreover, at the time of RSV bronchiolitis, the presence in nasal epithelia of increased levels of a Th2 cytokine (CCL5) involved in eosinophilic inflammation was associated with the increased likelihood of subsequent asthma (96). Finally, NPA levels of IL-10, a Th2 cytokine thought to play a critical role in post-RSV bronchiolitis airway hyperresponsiveness, were found to be higher in RSV-infected infants that later developed post-bronchiolitis wheeze (97). Viral infections could also adversely affect lung development as the inflammatory mediators, released during the acute phase, may negatively affect the remodeling process (98). In addition, possible persistence of a latent RSV infection may continually stimulate the host defense mechanisms (93). Numerous studies have emphasized the effects on airway tissues of the neurogenic inflammation (84). NGF and other neurotrophins, in addition to the many activities on nerve cells, play a role in inflammatory responses and tissue repair (84, 99). Through the persistent induction of neurogenic inflammation, RSV may be accountable for long-term remodeling of NANC innervation and for dysregulation of airway smooth muscle tone, leading to persistent bronchial hyperreactivity (84–87, 99).

Current antiviral approaches for RSV infection

Despite decades of research, there is no licensed RSV vaccine or effective therapeutic agent on the market, but currently, a large number of candidates are evaluated in preclinical phase or are undergoing clinical trials (100). Among the vaccine candidates in advanced clinical trials, particle and subunit vaccines are the most promising for pregnant women and the elderly, whereas subunit, live-attenuated, and vector-based vaccines are those apparently optimal for the pediatric population (101).

1 Particle-based vaccines include virus-like particles (VLPs), virosomes, and nanoparticles. VLPs contain non-replicating, non-infectious particles derived from virus-encoded proteins without immunosuppressive functions. Those under evaluation contain the RSV F protein, the RSV F protein in the post-fusion or pre-fusion conformation, and a chimeric protein formed by the RSV G and F proteins. Preliminary data in cellular and animal models suggest that they may be excellent stimulators of neutralizing antibodies and of the innate response, especially when combined with adjuvants (100). Virosome vaccines are reconstituted by virus envelope that lacks the genetic material and formulated with adjuvants or receptor ligands to enhance their immunogenicity. Two virosome vaccines incorporating, respectively, TLR-2 and TLR4 agonists were shown to induce systemic and mucosal immune responses in mice, similar to those induced by natural infection (100, 101). Nanoparticle vaccines include assemblies of synthesized polypeptides, presenting multiple copies of subunit antigens that can potentially mimic the size and shape of the natural

antigenic virus-encoded proteins. Phase I, II, and III clinical trials on RSV F nanoparticle vaccines, with or without adjuvants, are ongoing in adults, including healthy third-trimester pregnant women (100, 101).

- 2 Subunit vaccines, based on purified proteins isolated from RSV that have an elevated capacity to induce neutralizing antibodies, are in different phase clinical trials (95). These include the purified F protein, the central conserved region of the RSV-A G protein, a chimeric RSV F-G fusion protein, the CX3C central conserved region of the G protein, a post-fusion RSV F protein formulated with a synthetic TLR4 agonist, purified recombinant pre-fusion F protein, and extracellular domain of the SH protein (100–102).
- 3 Live-attenuated RSV vaccines must be characterized by a sufficiently attenuated viral replication to be safe in RSV-naïve infants but not too attenuated to eliminate an effective immune response with an adequate balance between safety and efficacy. The catastrophic failure of the 1960 formalin-inactivated RSV vaccine (FI-RSV) has been a barrier, for many years, to the development of new vaccine for many years. Indeed, FI-RSV vaccine not only failed to protect against wild-type RSV disease but also induced an exaggerated inflammatory response in infants RSV naïve before vaccination (103). Many children were hospitalized with LTRI, and, tragically, two infants died following naturally occurring RSV infection. The lack of protective effect was probably due to the formalin treatment which, altering the RSV membrane proteins, induced an exaggerated response lacking non-neutralizing antibody generation, immune complex deposition, complement activation, and Th2 response predominance (101–113). The cold-passaged two candidates MEDI-559- and the MEDI-559-derived RSVΔNS2Δ1313/1314 I are currently in phase I and phase II clinical trials. A phase II clinical trial of MEDI ΔM2-2, a vaccine that includes a deletion of the M2-2 gene, resulted in restricted viral shedding and high titers of RSV-neutralizing antibodies without illness. Two other candidates, the LIDΔM2-2 and D46cpΔM2-2 vaccines, sharing a similar M2-2 gene deletion, are undergoing phase I studies in RSV-seropositive children and RSV-seronegative infants and children (100–104).
- 4 Vector-based RSV vaccines include viral and bacterial vectors that have the capacity to mimic the natural infection of the host, inducing innate and adaptive responses. Promising results have been obtained with a simian adenovirus (PanAd3-RSV) and modified vaccinia virus Ankara (MVA-RSV) vectors expressing RSV F, N, and M2-1 proteins, with a vector vaccine composed of the Sendai virus vaccine, modified to express a full-length RSV F protein, with an alphavirus replicon particle encoding RSV F and G proteins and a recombinant baculovirus-based vaccine expressing the RSV G protein (100).

The development of next-generation agents, possible important tools in the management of RSV infection, has recently largely focused on three major target areas: the viral entry machinery, the viral RNA-dependent RNA-polymerase complex, and the viral component assembly (103). Of the many

RSV inhibitors described in recent years, only two orally available drug candidates have completed phase 2 clinical trials: GS-5806 and ALS-8176. GS-5806, an allosteric inhibitor of the RSV F protein, blocks viral entry, while ALS-8176, a nucleoside analog, competitively inhibits the RNA-dependent RNA-polymerase complex. Both compounds have shown decrease in viral load and disease symptoms in healthy volunteers challenged with the virus (111–105).

Conclusions

RSV infection is clinically confined to the respiratory epithelium in the vast majority of patients, yet extensive lung inflammation may cause life-threatening disease, particularly in high-risk populations. Despite its extremely simple structure, RSV possesses the ability to counteract host defense systems through complex mechanisms that facilitate viral replication

and progression and propagation of infection. The interplay between viral load and RSV genotype vis-a-vis the genetic and epigenetic characteristics of the host governs the magnitude of the injury to the lung and its impact on lung structure and function and the maturation of immune systems, and the latter with the potential of having repercussions of the infection, particularly in the form of protracted wheezing phenotypes, into late childhood years. A comprehensive understanding of the events involved in RSV pathogenesis and the interplay with host defenses acquired in recent years is starting to yield newly designed effective vaccines and antiviral therapies to prevent and control the infection of this major respiratory pathogen.

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